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A single-shot diagnostic platform based on copper nanoclusters coated with cetyl trimethylammonium bromide for determination of carbamazepine in exhaled breath condensate

Ayda Hatefi[a], Elaheh Rahimpour[b,*], Maryam Khoubnasabjafari[c], Maryam Edalat[d], Vahid Jouyban-Gharamaleki[e], Sima Alvani-Alamdari[f], Ali Nokhodchi[f,g], Mohammad Hossein Pournaghi-Azar[h], Abolghasem Jouyban[b,i]

[a] *Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[b] *Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[c] *Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[d] *Neuroscience Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[e] *Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[f] *Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran*
[g] *Pharmaceutics Research Laboratory, Arundel Building, School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK*
[h] *Electroanalytical Chemistry Laboratory, Faculty of Chemistry, University of Tabriz, Tabriz 5166616471, Iran*
[i] *Kimia Idea Pardaz Azarbayjan (KIPA) Science Based Company, Tabriz University of Medical Sciences, Tabriz 51664, Iran*

[*] Corresponding author: Rahimpour_e@yahoo.com

ABSTRACT

A fluorescent nanoprobe is designed for the determination of carbamazepine (CBZ) in exhaled breath condensate (EBC) of patients receiving CBZ. The probe consists of copper nanoclusters (Cu NCs) coated with cetyl trimethylammonium bromide. The interaction of probe with CBZ results in blocking non-radiative e^-/h^+ recombination defect sites on the surface of Cu NCs and consequently enhancing the fluorescence intensity of Cu NCs. The experimental conditions were optimized by using a response surface methodology (central composite design). Under the optimized conditions, the calibration plot is linear in the 0.2 to 20 μg mL^{-1} CBZ concentration range (excitation/emission wavelength: 290/480 nm) and the detection limit is as low as 0.08 μg mL^{-1}. The intra-day and inter-day relative standard deviations for six replicated measurements of 10 μg mL^{-1} CBZ are 3.9 % and 4.8 %, respectively. The method was applied for the determination of CBZ level in EBC of patients receiving CBZ. The accuracy of the method was confirmed by HPLC-UV analysis as a references method.

Keywords: Carbamazepine; Copper; Nanoprobe; Nanocluster; Fluorescence; Response surface methodology; central composite design;

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1. Introduction

Carbamazepine (5H-dibenzo [b,f] azepine-5-carboxamide, CBZ) is a carboxamide derivative of iminostilbene [1] and widely used for the therapy of patients with partial and generalized tonic-clonic epilepsy and psychiatric diseases [2]. So far, various methods, such as liquid chromatography–electrospray ionization mass spectrometry method (LC-EIA-MS) in serum samples [3], LC–MS-MS method in whole blood, serum and plasma [4], ion mobility spectrometry in formulation samples [5], stacking capillary electrophoresis method in serum samples [6], high-performance liquid chromatography-diode array detection assay after a simple sample preparation method by a packed sorbent in plasma samples [7], online solid phase extraction - LC coupled with high resolution mass spectrum under targeted MS/MS analysis mode in serum sample [8], and a gas chromatography method [9] have been developed for the determination of CBZ. Although the advantages of such methods are undeniable, most of these techniques need a sample preparation procedure to remove matrix components or a derivatization step before analysis and also a labelled internal standard. So, development of an easy-to-use method that can be applied for off- and on-line determination of drugs concentrations in biological samples has always been of interest in bioanalytical and clinical applications. It is important to note that the assay of biomolecules should be fast, cost-effective, selective, and sensitive. For this purpose, increasing attention has been focused on the construction of fluorescent nanoprobes for drugs [10]. Quantum dots (QDs) and nanoclusters (NCs) with particular photophysical and photochemical properties, such as high luminescence intensity, stability and efficiency, narrow, and tunable spectrum according to their size and material composition, good solubility and biocompatibility, low toxicity, can offer significant advantages

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for biosensing [11, 13]. However, to the best of our knowledge, QDs and/or NCs as an optical nanoprobe are rarely employed in the determination of CBZ and there is only one report in the literature for the detection of CBZ. Ma *et al.* [14] developed a fluorescent probe based on N-doped carbon dots and used to the determination of CBZ in tablet and human urine spiked samples. They illustrated that the presence of CBZ in the range of 10–90 μg mL^{-1} result in fluorescence quenching of QDs. The detection limit was 1.93 μg mL^{-1} [14].

Exhaled breath condensate (EBC) as a biological sample was chosen for this study since it possesses a number of advantages over other biological samples [15]. The collection of EBC, which has been used since the 1980s [16], are very simple and repeatable method without any side effect or any appreciable discomfort for the EBC sample donors [17]. EBC also have a few number of interfering compounds in compared with other matrices including blood, plasma, sputum, and urine [18]. Furthermore, the EBC collection can be repeated as often as required without any variation in the fluids on the lung surfaces [19]. The analysis of drugs in EBC is a promising process that has attracted a lot of attention [20-22].

Herein, to continue our research works on EBC [20-22], we synthesize Cu NCs coated with cetyl trimethylammonium bromide (CTAB) and use them for the developing an effective nanoprobe with good selectivity and sensitivity for determination of CBZ level in EBC samples. Detection mechanism is based on the blocking non-radiative e^-/h^+ recombination defect sites on the surface of the NCs and improving the fluorescence intensity of NCs proportional with CBZ adding. For reaching better analytical figures of merit for the nanoprobe, the experimental conditions were investigated by response surface methodology (RSM), which employs statistical methods to present a second-order polynomial model between independent variables and a response variable.

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2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and ultrapure deionized water was obtained from Ghazi Pharmaceutical Co. (Tabriz, Iran, www.sgco-infusion.com) employed throughout the measurements. (Cu $(NO_3)_2{\cdot}3H_2O$, Sigma-Aldrich, www.sigmaaldrich.com), CTAB ($C_{19}H_{42}BrN$, Merck, www.merck.com), citric acid ($C_6H_8O_7{\cdot}H_2O$, Merck) and hydrazine hydrate ($H_4N_2.H_2O$, Sigma-Aldrich, www.sigmaaldrich.com) were used in the CuNCs synthesis procedure. Sodium chloride (NaCl, Scharlau, www.scharlab.com), potassium chloride (KCl, Merck), sodium sulfate (Na_2SO_4, Merck), aluminium nitrate ($Al(NO_3)_3{\cdot}9H_2O$, Merck), magnesium nitrate ($Mg(NO_3)_2{\cdot}6H_2O$, Chem-lab, www.chem-lab.be), sodium carbonate (Na_2CO_3, Merck), iron chloride ($FeCl_3{\cdot}6H_2O$, Merck), calcium oxalate ($CaC_2O_4{\cdot}H_2O$, Carl. Roth GmbH & Co., www.carlroth.com/en/en), glucose (Merck), lamotrigine (Arastoo Pharmaceutical Company, Iran, www.arasto.com), sodium valproate (Temad Pharmaceutical Co., Iran, www.temad.com/fa), phenytoin (Amin Pharmaceutical Company, Iran, www.aminpharma.com) and phenobarbital (Pars Darou company, Iran, www.parsdarou.ir) were used in the interference study. A solution of 1000 mg L^{-1} CBZ was prepared by dissolving a proper amount in methanol. Different concentrations of CBZ were prepared by diluting standard stock solutions with deionized water.

2.2. Apparatus and software

Fluorescence intensity were measured at 25 ˚C on a FP-750 spectrofluorometer (Jasco Corp., Japan, www.jasco.co.jp) with 20 nm band-pass in both of the excitation and emission paths and the sensitivity of medium. The instrument have a 150 W xenon lamp, dual monochromators, 1.0

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cm quartz cell, Peltier thermostated single cell holder model ETC-272 (JASCO Corp., Japan), and supported with PC–based Windows® Spectra Manager TM software for JASCO Corporation. A double-beam UV–Vis spectrophotometer model UV-1800 (Shimadzu, Japan, www.shimadzu.com) with 1.0 cm quartz cells was used to record the UV–Vis absorption spectra. The pH adjustments of the used solutions were controled using a digital pH-meter model 744 (Metrohm Ltd., Switzerland, www.metrohm.com) supplied with a glass-combined electrode. An ultrasonic bath (Alex machine, Istanbul) and An electronic analytical balance model AB204-S (Mettler Toledo, Switzerland, www.mt.com) was employed in this study. The shape and size of prepared QDs were characterized by a transmission electron microscopy model CM30 (Philips, The Netherlands, www.philips.com).

Design and analysis of the central composite experiments were performed in MINITAB (Minitab Inc. Release 17.0, www.minitab.com/en-us/products/minitab) statistical package.

2.3. Preparation of Cu NCs

The Cu NCs were synthesized by following the method reprted in literature [23]. 10 mL of 2 mol L^{-1} aqueous solution of hydrazine hydrate containing 0.5 mmol L^{-1} citric acid (0.05 mL citric acid of 0.1 mol L^{-1}) and 5 mmol L^{-1} CTAB (0.0182 gr) were stirred for 30 min and added to 10 mL of 20 mmol L^{-1} solution of $Cu(NO_3)_2.3H_2O$ (0.0482 gr) containing 0.5 mmol L^{-1} citric acid (0.05 mL citric acid of 0.1 mol L^{-1}) and 5 mmol L^{-1} CTAB (0.0182 gr). The obtained mixture was agitated at room temperature for 3 h. (further details are given in the Electronic Supporting Material (ESM)) The prepared NCs were stored at 4°C in a dark place. The concentration of the prepared CuNCs solution was determined as 2.5 mg L^{-1} according to the flame atomic absorption spectroscopy used in the current study.

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2.4. Sample preparation

EBC samples were collected by a lab-made cooling trap system [24]. EBC samples used for method validation were a pool of samples collected from healthy subjects. EBC samples were directly analyzed without any pretreatment. Patient EBC samples were collected from four patients after oral administration of CBZ. Sample donors signed a consent form approved by the Ethics Committee of National Institute for Medical Research Development (approved No. IR NIMAD REC 1396 356).

2.5. General procedure

Fluorescence detection was made in a 2 mL vial by a batch method. In brief, 50 µL of 0.1 mol L^{-1} phosphate buffer (pH 5.0), and 50 µL of 0.5 mg L^{-1} Cu-NCs were added into the vial. Afterwards, a proper volume of CBZ standard solution in the range of 0.2 to 20 µg mL^{-1} was added and the volume was set in 0.5 mL with deionized water. To prevent a matrix effect on the validation data, EBC samples were used to validate the fluorimetric determination method. For this purpose, the EBC samples were collected from healthy subjects, spiked with proper aliquots of standard CBZ solution. After 10 min sonication, the fluorescence intensity was recorded at λ_{em}= 480 nm after excitation at 290 nm and used as an analytical signal.

2.5. Real sample analysis

EBC samples was obtained from four subjects who had taken CBZ. 0.25 mL of EBC samples directly (without any preparation) added into a 2 mL vial containing 50 µL of 0.1 mol L^{-1} phosphate buffer (pH 5.0), and 50 µL of 0.5 mg L^{-1} Cu-NCs. The volume in vial was adjusted to

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0.5 mL with deionized water. Then, the solution was equilibrated for 10 min in the sonication bathat room temperature. Finally, the obtained solution was transferred into microcell and the fluorescence intensity was measured at 480 nm after excitation at 290 nm. CBZ levels in EBC of patients were also analyzed using HPLC method described in the literature [25].

3. Results and discussion

3.1. Choice of nanoparticle's type

In order to investigate the nanoparticle's type, various nanomaterials such as Cu NCs, Zn NCs, Ag NCs and carbon QDs (CQDs) were synthesized and used for determination of CBZ in a similar condition. Fluorescence spectra of these nanomaterials in the absence and presence of CBZ (10 μg mL^{-1}) are shown in Fig. 1S. As can be seen, Ag NCs, Zn NCs, CQDs do not any spectral changes in the presence of CBZ and only a slight interaction is observed between Cu NCs and CBZ. According to these results, in the next attempt, CTAB modified Cu NCs was used for production of an effective interaction between nanoparticles and CBZ due to non-ionic property of CBZ. The possible reason for such a large variation in fluorescence spectra of CTAB modified Cu NCs (Fig. 2S) is related to the incorporation of CBZ into the inner hydrophobic part of produced ad-micelles or hemi-micelles of CTAB on the surface of NCs and changing in environmental conditions of them that produce a suitable assemble and change the intrinsic fluorescence of nanoparticles.

3.2. Characterization of Cu-NCs

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The shape and size of synthesized NCs were characterized by transmission electron microscopy (TEM). As can be seen in Fig. 1, the NCs are uniform in size and exhibit a nearly spherical shape with an average size of <8 nm.

(Fig. 1 here)

3.3. Detection mechanism discussion

In the high concentration of prepared NCs and under single wavelength excitation at 290 nm, the Cu-NCs shows two fluorescent bands centred at 415 and 480 nm (Fig. 2). According to previous studies, the dual fluorescent bands of the QDs can be attributed to different surface states [26]. It should be noted that the low concentration of NCs was used for detection of CBZ; thus, in the absence of CBZ, the synthesized NCs emits only a well-resolved emission peak at 415 nm. Upon the addition of CBZ, the fluorescence spectra show a new emission peak centred at 480 nm, whereas the intensity at 415 nm of the NCs remains relatively unchanged. The fluorescence spectra of the designed nanoprobe after addition of various concentration of CBZ is shown in Fig. 3. As can be seen, the fluorescence intensity is proportional to the amount of CBZ, ranging from 0.2 to 20 μg mL^{-1}, which can be used for the quantification of CBZ.

Two classes of fluorescence emission mechanisms have been reported for QDs and/or NCs in the literature: (i) the bandgap transitions; (ii) transitions arising from surface defects in QDs; generally any sites that result in surface energy traps [27]. Surface modification of QDs and interaction with surrounding species may change their chemical and optical properties [28, 29] and cause to some effects including: (i) activation of fluorescence by enhancement of excitonic and defect emission by blocking non-radiative electron/ hole (e^-/h^+) recombination defect sites on the surface of the QDs, (ii) creation of new traps on the surface of the QDs causing appearance of new emission peaks, (iii) enhancement of the selectivity and efficiency of light-induced

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reactions done on the surface of QDs [30], (iv) it may quench QDs fluorescence through two possible pathways: fluorescence resonance energy transfer (FRET) and the common charge transfer [31].

In this study, CTAB was grafted on the Cu-NCs surface and served as the linking reagent for linking of CBZ. We conjectured that the interaction of Cu-NCs with CBZ resulted in blocking non-radiative e^-/h^+ recombination defect sites on the NCs surface and consequently enhancing the fluorescence intensity of NCs.

(Fig. 2 and 3 here)

3.4. Optimization of reaction conditions by a central composite design (CCD)

RSM was used to investigate the influence of several reaction factors that affect the determination of CBZ by considering the interactive effects of independent factors (pH, the probe and phosphate buffer concentration, and the reaction time of the system, in this case). Respective data and figures are given in ESM. The optimum values obtained for each investigated variable to reach the maximum signal intensity are: pH 6.0, [phosphate buffer]=16.5 mmol L^{-1}, [Cu-NCs]= 0.05 mg L^{-1}, and time = 10 min.

3.5. Interferences study

The selectivity of the nanoprobe toward analyte in the presence of various anions, cations and possible co-administered drugs is also investigated by measuring the fluorescence intensity upon the addition of various concentrations of interferents. From a practical viewpoint, CBZ is used as a single drug the treatment of most forms of epilepsy and the drug of first choice in the germinal neural-gia [32] and as a combined drug regimen in bipolar mania, restless legs syndrome and schizophrenia [33]. The most frequently co-prescribed drugs with CBZ include

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231 the antiepileptic drugs and we tested the interference of available antiepileptic drugs in this work.
232 The results are presented in Table 1. The results show that the presence of anions and cations,
233 such as Na^+, Cl^-, SO_4^{2-}, K^+ and Ca^{2+} do not affect the fluorescence intensity of the nanoprobe.
234 The tolerance concentrations of other substances (with the exception of Fe^{3+} and lamotrigine)
235 when determination 10 $\mu g.mL^{-1}$ of CBZ by employing the nanoprobe are at least 10 times higher
236 than that of CBZ. However, the amounts of lamotrigine in EBC samples are reported to be in the
237 range of 0.5 – 0.7 $\mu g.mL^{-1}$ [21] which are below its tolerable level. So, there would be no
238 interference from it in CBZ determination in EBC samples.

239 *(Tables 1 here)*

240 In addition, patients receiving CBZ, may take some non-prescribed over-the-counter drugs
241 such as pain killers etc. The interference of these drugs were also investigated. But this time
242 according to detection range founded for CBZ in EBC (0.3 – 0.5 $\mu g.mL^{-1}$), interference studies
243 were performed for CBZ concentration of 0.5 $\mu g.mL^{-1}$. The results are shown in Table 2. As can
244 be seen naproxen, chlordiazepoxide, glibenclamide, oxazepam, acetaminophen, celecoxib, and
245 clonazepam interfere with CBZ determination when they are present in 25 times of the
246 concentration rations of interfering substance to CBZ. It means that interfering concentration for
247 these drugs is about 12.5 $\mu g.mL^{-1}$ which is much higher than concentration founded for the drugs
248 in EBC. As limited data are published for concentration range of drugs in EBC samples, we
249 checked this claim for a safe drug e.g. acetaminophen. For this purpose, a healthy volunteer is
250 given 1 g of acetaminophen daily for 3 days. After this time, the concentration of acetaminophen
251 in EBC of investigated subject is found to be < 0.1 $\mu g.mL^{-1}$ which is below its tolerable level.
252 These results demonstrate that the presented nanoprobe has good selectivity for CBZ detection.

253 *(Tables 2 here)*

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3.6. Analytical figures of merit

In the optimum conditions described, a linear relationship (R^2 =0.993) between the CBZ concentration and fluorescence intensity was observed at the concentration range of 0.2−20 µg mL^{-1} (excitation/emission wavelength: 290/480 nm). The regression equation was ΔI_F = 21.626 C_{CBZ} + 18.691, where ΔI_F is the response intensity difference between blank (nanoprobe in the absence of CBZ) and sample solution (nanoprobe in the presence of CBZ) in an arbitrary unit, and C_{CBZ} is the concentration of CBZ in µg mL^{-1} (Fig. 4). The detection limit of the present method for CBZ determination was computed after the employig of the mentioned general procedure for blank solutions. The detection limit and quantification limit, defined as $3\ S_b/m$, and $10\ S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration plot) are 0.08 µg mL^{-1} and 0.20 µg mL^{-1}, respectively. The precision of the method was investigated by replicating the CBZ determination during the course of experimentation on different days and on the same day. The intra-day and inter-day relative standard deviations (%*RSD*) for six replicated measurements of 10 µg mL^{-1} CBZ are 3.9 % and 4.8 %, respectively. Furthermore, the influence of Cu-NCs got from different batches on the analytical response was also investigated. The %*RSD* (n = 3) from fluorescence signals was 5.2 %, demonstrating the good batch-to-batch reproducibility of the synthesized NCs. A comparison of our method with some other published methods for determination of CBZ is shown in Table 3. As can be seen, the LOD of method is good and comparable with that of other reported methods. Also, the method is relatively rapid due to direct analysis and no need for any sample preparation procedure prior to analysis. Whereas, most of reported method applied for determination of CBZ used a liquid or solid-phase extraction for cleanup and analyte preconcentration before the sample analysis. These results show that the presented nanoprobe has good performance for CBZ determination. However,

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there are some issues related to the used nanoprobe and EBC which limit their application. These issues include (i) poor storage stability of nanoprobe over longer periods of time due to their small size (with an average size of <8 nm) and large surface area which can lead to particle-particle aggregation and making physical handling of nanoparticles difficult in liquid form; and (ii) some limitations associated with the EBC samples including adsorption of analytes to the surface of the collection setup, patient-to-patient variability in EBC volume and the effect of lung function on the collected sample volume and unavailability of an accepted dilution marker for EBC samples. These limitations and some solutions to overcome them are discussed in reference [15] in details.

(Fig. 4 here)

(Table 3 here)

3.7. EBC sample analysis

To further investigate the applicability of the nanoprobe on real samples, the probe was used to detect CBZ in EBC of patients receiving CBZ. All samples were taken 1 h after taking the last dose. The results of the analysis are summarized in Table 4. The accuracy of the method was approved by HPLC measurement as a reference method applied on a same EBC sample [34]. Using the paired *t*-test (2-tail) shows no significant difference at 95% confidence level demonstrating the method is accurate and has a great potential for determination of CBZ in EBC samples.

(Table 4 here)

4. Conclusions

In this study, a sensitive nanoprobe for CBZ determination has been developed. The nanoprobe takes advantage of the high sensitivity and the high stability of the CTAB-coated NCs

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toward CBZ to provide an effective platform for reliable determination of CBZ. The nanoprobe was successfully used for the determination of CBZ in EBC of patients receiving CBZ and an average level of 0.47 μg mL^{-1} was reported. Reliability and no practical limitation of the method makes it a promising system for routine quantification of CBZ in clinical practices. This is the first report on CBZ levels in EBC and these types of CBZ quantifications using a non-invasive sampling method are highly in demand in dosage adjustment of CBZ.

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References

[1] Kutt H, Paris-Kutt H (1982) Antiepileptic Drugs. In: Woodbury DM, Penry JR, Pippenger CE (Eds.). Raven Press, New York, pp 453.

[2] Duche P, Loiseau B (1995) Antiepileptic Drugs. In: Levy RH, Mattson RH, Meldrum BS, Penry JK, Dreyfuss FE (Eds.) 4rd edn. Raven Press, New York, pp 555.

[3] Breton H, Cociglio M, Bressolle F, Peyriere H, Blayac JP, Hillaire-Buys D (2005) Liquid chromatography–electrospray mass spectrometry determination of carbamazepine, oxcarbazepine and eight of their metabolites in human plasma. J. Chromatogr. B 828:80-90.

[4] Deeb S, McKeown DA, Torrance HJ, Wylie FM, Logan BK, Scott KS (2014) Simultaneous analysis of 22 antiepileptic drugs in postmortem blood, serum and plasma using LC–MS-MS with a focus on their role in forensic cases. J. Anal. Toxicol. 38:485-494.

[5] Kalhor H, Hashemipour S, Yaftian MR, Shahdousti P (2016) Determination of carbamazepine in formulation samples using dispersive liquid–liquid microextraction method followed by ion mobility spectrometry. Int. J. Ion. Mobil. Spectrom. 19:51-56.

[6] Lin Y-Y, Wang C-C, Ho Y-H, Chen C-S, Wu S-M (2013) Analysis of carbamazepine and its five metabolites in serum by large-volume sample stacking–sweeping capillary electrophoresis. Anal. Bioanal. Chem. 405:259-266.

[7] Ferreira A, Rodrigues M, Oliveira P, Francisco J, Fortuna A, Rosado L, Rosado P, Falcão A, Alves G (2014) Liquid chromatographic assay based on microextraction by packed sorbent for therapeutic drug monitoring of carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, phenytoin and the active metabolites carbamazepine-10, 11-epoxide and licarbazepine. J. Chromatogr. B 971:20-29.

[8] Qu L, Fan Y, Wang W, Ma K, Yin Z (2016) Development, validation and clinical application of an online-SPE-LC-HRMS/MS for simultaneous quantification of phenobarbital, phenytoin, carbamazepine, and its active metabolite carbamazepine 10, 11-epoxide. Talanta 158:77-88.

[9] Burke J, Thenot J (1985) Determination of antiepileptic drugs. J. Chromatogr. B 340:199-241.

[10] Wang Y, Yan B, Chen L (2012) SERS tags: novel optical nanoprobes for bioanalysis. Chem. Rev. 113:1391-1428.

[11] Gill R, Zayats M, Willner I (2008) Semiconductor quantum dots for bioanalysis. Angew. Chem. Int. Ed. 47:7602-7625.

[12] Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nature Biotechnol. 21:41-46.

ACCEPTED VERSION

[13] Alivisatos P (2004) The use of nanocrystals in biological detection. Nature Biotechnol. 22:47-52.

[14] Ma Y, Song Y, Ma Y, Wei F, Xu G, Cen Y, Shi M, Xu X, Hu Q (2018) N-doped carbon dots as a fluorescent probe for the sensitive and facile detection of carbamazepine based on the inner filter effect. New J. Chem. 42:8992-8997.

[15] Khoubnasabjafari M, Rahimpour E, Jouyban A (2018) Exhaled breath condensate as an alternative sample for drug monitoring. Bioanalysis 10:61-64.

[16] Hunt J (2002) Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. J. Allergy Clin. Immunol. 110:28-34.

[17] Chow S, Yates D, Thomas P (2008) Reproducibility of exhaled breath condensate markers. Eur. Respir. J. 32:1124-1126.

[18] De Paiva MJN, Menezes HC, De Lourdes Cardeal Z (2014) Sampling and analysis of metabolomes in biological fluids. Analyst 139:3683-3694.

[19] Effros RM, Biller J, Foss B, Hoagland K, Dunning MB, Castillo D, Bosbous M, Sun F, Shaker R (2003) A simple method for estimating respiratory solute dilution in exhaled breath condensates. Am. J. Respir. Crit. Care Med. 168:1500-1505.

[20] Khoubnasabjafari M, Ansarin K, Jouyban-Gharamaleki V, Panahi-Azar V, Hamidi S, Azarmir Z, Jouyban A (2017) Methadone concentrations in exhaled breath condensate, serum and urine of patients under maintenance treatment. Iran J. Pharm. Res. 16:1621-1630.

[21] Jouyban A, Samadi A, Khoubnasabjafari M, Jouyban-Gharamaleki V, Ranjbar F (2017) Amidosulfonic acid-capped silver nanoparticles for the spectrophotometric determination of lamotrigine in exhaled breath condensate. Microchim. Acta 184:2991-2998.

[22] Mohamadian E, Shayanfar A, Khoubnasabjafari M, Jouyban-Gharamaleki V, Ghaffary S, Jouyban A (2017) Analysis of deferiprone in exhaled breath condensate using silver nanoparticle-enhanced terbium fluorescence. Analy. Methods 9:5640-5645.

[23] Ghosh SK, Rahman DS, Ali AL, Kalita A (2013) Surface plasmon tunability and emission sensitivity of ultrasmall fluorescent copper nanoclusters. Plasmonics 8:1457-1468.

[24] Jouyban A, Khoubnasabjafari M, Ansarin K, Jouyban-Gharamaleki V (2013) Breath sampling setup. Iranian patent 81363.

[25] Karami-Bonari AR, Tamizi E, Samini M, Jabbaribar F, Jouyban A (2009) HPLC method for simultaneous determination of five antiepileptic drugs in rat plasma, Asian J. Chem. 21(8): 6501-6507.

[26] Qu S, Chen H, Zheng X, Cao J, Liu X (2013) Ratiometric fluorescent nanosensor based on water soluble carbon nanodots with multiple sensing capacities. Nanoscale 5:5514-5518.

ACCEPTED VERSION

[27] Lim SY, Shen W, Gao Z (2015) Carbon quantum dots and their applications. Chem. Soc. Rev. 44:362-381.

[28] Weller H (1993) Colloidal semiconductor q-particles: chemistry in the transition region between solid state and molecules. Angew. Chem. Int. Ed. Engl. 32:41-53.

[29] Kamat PV (1993) Photochemistry on nonreactive and reactive (semiconductor) surfaces. Chem. Rev. 93:267-300.

[30] Isarov AV, Chrysochoos J (1997) Optical and photochemical properties of nonstoichiometric cadmium sulfide nanoparticles: surface modification with copper (II) ions. Langmuir 13:3142-3149.

[31] Fan L, Hu Y, Wang X, Zhang L, Li F, Han D, Li Z, Zhang Q, Wang Z, Niu L (2012) Fluorescence resonance energy transfer quenching at the surface of graphene quantum dots for ultrasensitive detection of TNT. Talanta 101:192-197.

[32] Bertilsson L, Tomson T (1986) Clinical pharmacokinetics and pharmacological effects of carbamazepine and carbamazepine-10, 11-epoxide, Clin. Pharmacokinet 11(3): 177-198.

[33] Severus E, Fiebig J, Pfennig A, Bauer M, (2018) Carbamazepine and valproic acid as mood stabilizers in the therapy of bipolar disorder. Is their use still justified? Psycopharmakotherapie, 25: 247-249.

[34] Rockville M (2007) The United States Pharmacopoeia 30, the National Formulary 25 US Pharmacopeial Convention, Electronic version.

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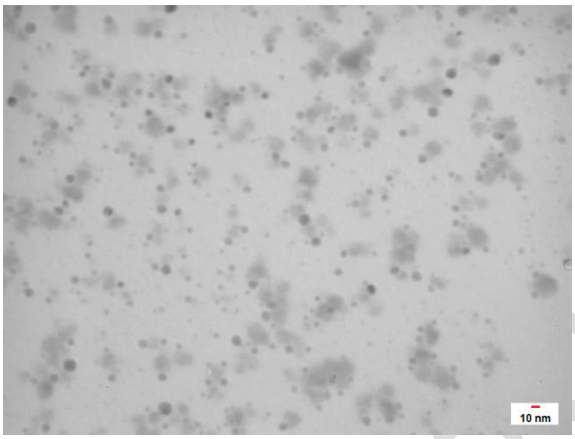

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403 **Fig. 1.** TEM image CTAB coated Cu-NCs.

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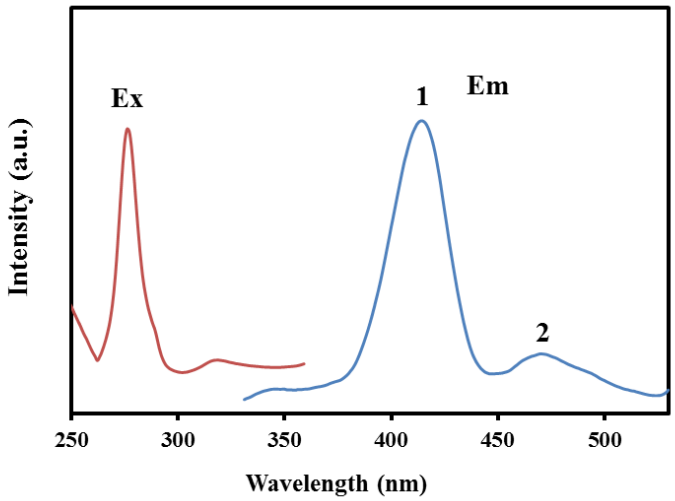

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408 **Fig. 2.** Excitation and emission spectrum of CTAB coated Cu NCs

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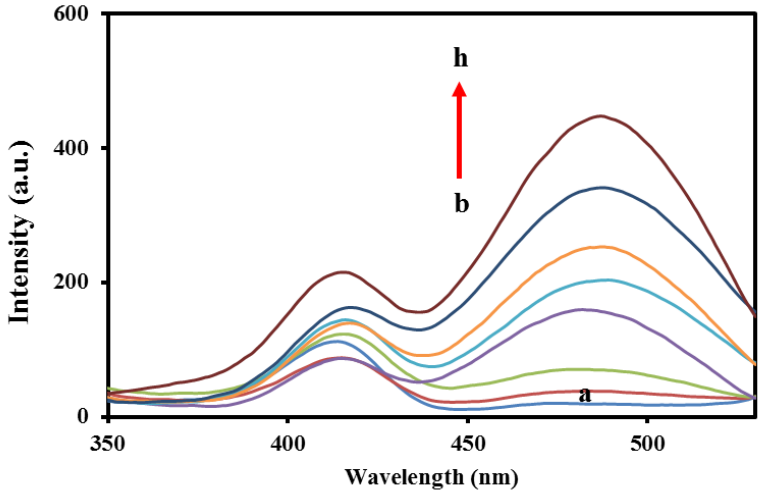

Fig. 3. Fluorescence spectrum of the CTAB coated Cu NCs in the absence (a) and presence of CBZ in the concentration range of 0.2–20 µg mL^{-1} (b-h). Conditions: pH 6, [phosphate buffer]=16.5 mmol L^{-1}, [Cu-NCs]= 0.05 mg L^{-1}, and time = 10 min, $\lambda_{ex}/\lambda_{em}$: 290/ 480 nm.

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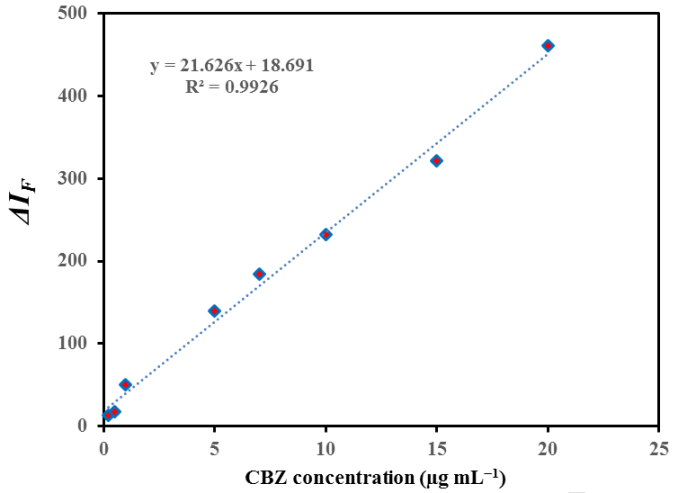

Fig. 4. Fluorescence intensity difference (ΔI_F) of CTAB coated Cu NCs in the absence and presence of CBZ versus CBZ concentration in the range of 0.2–20 µg mL^{-1}. Conditions: pH 6, [phosphate buffer]=16.5 mmol L^{-1}, [Cu-NCs]= 0.05 mg L^{-1}, and time = 10 min, $\lambda_{ex}/\lambda_{em}$: 290/ 480 nm.

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Table 1
Tolerance limits of interfering substances (commonly existing compounds in biological samples, co-prescribed drugs) in the determination of 10 µg mL^{-1} of CBZ.

Interfering substances	Tolerance limit
Na^{+}, Cl^{-}, SO_4^{-2}	1000
K^{+}	700
Oxalate	500
Ca^{+2}, Glucose, Sucrose	200
Mg^{2+}, CO_3^{2-}, Sodium valproate	100
Al^{3+}, Phenobarbital, Phenytoin, Topiramate	10
Fe^{3+}, Lamotrigine	1

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Table 2
Tolerance limits of some co-administrated and over-the-counter drugs in the determination of 0.5 μg mL^{-1} of CBZ.

Interfering substances	Tolerance limit
Cetirizine	500
Sitagliptin, Dextromethorphan	200
Nifedipine, Amiloride, Amoxicillin, Alprazolam, Lovastatin, Metoprolol, Nicotinamide, Ibuprofen, Pantoprazole, Diltiazem, Diazepam, Salicylic acid	100
Naproxen, Chlordiazepoxide, Glibenclamide, Oxazepam, Acetaminophen, Celecoxib, Clonazepam	25

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Table 3
Comparison of analytical characteristics of the presented method with other reported techniques for determination of CBZ.

Method	Clean up/Pre-concentration method	Real sample	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	Reference
LC-EIA-MS[a]	Protein precipitation extraction	Serum	0.5 – 20	–	[3]
LC–MS-MS	Protein precipitation extraction	Whole blood, serum and plasma	0.5 – 50	0.25	[4]
Ion mobility spectrometry	DLLME[b]	Formulation samples	0.05 – 10	0.025	[5]
SCE[c]-UV	LLE[d]	Serum	0.03 – 25	0.01	[6]
HPLC-DAD[e]	MEPS[f]	Plasma	0.1 – 15	–	[7]
N-doped carbon dots based fluorescent probe	–	Tablet and human urine	10–90	1.93	[14]
CTAB coated Cu NCs-based fluorimetric assay	–	EBC	0.2 – 20	0.08	This work

[a] Liquid chromatography–electrospray mass spectrometry
[b] Dispersive liquid–liquid microextraction
[c] Stacking capillary electrophoresis
[d] Liquid–liquid extraction
[e] High-performance liquid chromatography–diode-array
[f] Microextraction by packed sorbent
[g] High resolution mass spectrum
[h] Solid phase extraction

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Table 4

Details of the real samples and found concentration of CBZ by our method and HPLC method in the EBC samples of patients treated with CBZ

No.	Gender	Age (year)	Daily dosage (mg)	CBZ level in EBC (μg mL^{-1})		*t*-value[b]
				Present method[a]	References method[a]	
1	Female	32	500	0.51 ± 0.02	0.52 ± 0.04	0.625
2	Male	61	400	0.39 ± 0.05	0.42 ± 0.01	1.11
3	Male	39	600	ND[c]	ND	–
4	Female	19	800	0.50 ± 0.02	0.55 ± 0.08	1.14

[a]The found values are the averages of three determinations ± standard deviation in μg mL^{-1}.
[b]Critical *t*-value is 4.30.
[c] Not detected.

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